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Standard RT-PCR to Amplify the SH Gene of Mumps Virus

Use of trade names or commercial sources is for identification only and does not imply endorsement by CDC.

Primers for Mumps (PCR product will be 675 nucleotides):
Forward SH1, 5’ AGT AGT GTC GAT GAT CTC AT 3’
Reverse SH2, 5’ GCT CAA GCC TTG ATC ATT GA 3’

This RT-PCR assay can be used to detect mumps RNA in clinical samples or in infected cell culture with the caveat that this assay is less sensitive than real time RT-PCR. The sequence of the PCR product containing the coding region of the SH gene can be used to determine the viral genotype (see Jin et al,. Arch Virol. 2005;150:1903-9).
Primer sequences are from: Jin L, Beard S, Brown DWG. Genetic heterogeneity of mumps virus in the United Kingdom: identification of two new genotypes. J Infect Dis 1999; 180:829 33.
Mumps SH gene. The sequence of the 675 nucleotide amplicon is shown below as cDNA. The primer sequences are in italics and the 318 nt. SH gene is underlined. The SH protein is also shown.

SH1

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     AGTAGTGTCGATGATCTCATCAGGTACTAATCTTAGATTGGTGATTCGTCCTGCAATTTT
   1 ---------+---------+---------+---------+---------+---------+ 60
     TCATCACAGCTACTAGAGTAGTCCATGATTAGAATCTAACCACTAAGCAGGACGTTAAAA

     AAAAGATTTAGAAAAAAACTAAAATAAGAATGAATCTCCTAGGGTCGTAACGTCTCGTGA
  61 ---------+---------+---------+---------+---------+---------+ 120
     TTTTCTAAATCTTTTTTTGATTTTATTCTTACTTAGAGGATCCCAGCATTGCAGAGCACT

     CCCTGCCGTCGCACTATGCCGGCAATCCAACCTCCCTTATACCTAACATTTCTAGTGCTA
 121 ---------+---------+---------+---------+---------+---------+ 180
     GGGACGGCAGCGTGATACGGCCGTTAGGTTGGAGGGAATATGGATTGTAAAGATCACGA

a                    M  P  A  I  Q  P  P  L  Y  L  T  F  L  V  L   -

     ATCCTTCTCTATCTCATCATAACCCTGTATGTCTGGACTATATTGACTATTAACTATAAG
 181 ---------+---------+---------+---------+---------+---------+ 240
     TAGGAAGAGATAGAGTAGTATTGGGACATACAGACCTGATATAACTGATAATTGATATTC

a       I  L  L  Y  L  I  I  T  L  Y  V  W  T  I  L  T  I  N  Y  K   -

     ACGGCGGTGCGATATGCAGCACTGTACCAGCGATCCTTCTCTCGCTGGGGTTTTGATCAC
 241 ---------+---------+---------+---------+---------+---------+ 300
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        TGCCGCCACGCTATACGTCGTGACATGGTCGCTAGGAAGAGAGCGACCCCAAAACTAGTG

a       T  A  V  R  Y  A  A  L  Y  Q  R  S  F  S  R  W  G  F  D  H   -

        TCACTCTAGAAAGATCCCCAATTAGGACAAGTCCCGATCCGTCACGCTAGAACAAGCTGC
    301 ---------+---------+---------+---------+---------+---------+ 360
        AGTGAGATCTTTCTAGGGGTTAATCCTGTTCAGGGCTAGGCAGTGCGATCTTGTTCGACG

a       S  L  *

        ATTCAAATGAAGCTGTGCTACCATGAGACATAAAGAAAAAAGCAAGCCAGAACAAACCTA
    361 ---------+---------+---------+---------+---------+---------+ 420
        TAAGTTTACTTCGACACGATGGTACTCTGTATTTCTTTTTTCGTTCGGTCTTGTTTGGAT


        GGATCATAACACAATACAGAATATTAGCTGCTATCACAACTGTGTTCCGGCCACTAAGAA
    421 ---------+---------+---------+---------+---------+---------+ 480
        CCTAGTATTGTGTTATGTCTTATAATCGACGATAGTGTTGACACAAGGCCGGTGATTCTT


        AATGGAGCCCTCGAAACTATTTATAATGTCGGACAATGCCACCTTTGCACCTGGACCTGT
    481 ---------+---------+---------+---------+---------+---------+ 540
        TTACCTCGGGAGCTTTGATAAATATTACAGCCTGTTACGGTGGAAACGTGGACCTGGACA


        TGTTAATGCGGCTGGTAAGAAGACATTCCGAACCTGTTTCCGAATATTGGTCCTATCTGT
    541 ---------+---------+---------+---------+---------+---------+ 600
        ACAATTACGCCGACCATTCTTCTGTAAGGCTTGGACAAAGGCTTATAACCAGGATAGACA


        ACAAGCAGTTATCCTTATATTGGTTATTGTCACTTTAGGTGAGCTTATTAGGATGATCAA
    601 ---------+---------+---------+---------+---------+---------+ 660
        TGTTCGTCAATAGGAATATAACCAATAACAGTGAAATCCACTCGAATAATCCTACT AGTT

        TGATCAAGGCTTGAGC
    661 ---------+------ 676
        ACTAGTTCCGAACTCG (SH2R)
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Superscript III RT-PCR Protocol for Mumps SH Gene Amplification

This is a standard RT-PCR reaction. This is a one-tube reaction, so there is a minimum of specimen handling. Some points to consider:

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1. Controls.

Positive control should be synthetic mumps SH gene RNA with an insert. The presence of the insert leads to a larger amplicon for the control RNA than for RNA isolated from clinical specimens. This difference in size is visible in agarose gels and can be used to recognize contamination of clinical sample RNA with control RNA. See sizes of PCR products in appendix 3.

The positive control can be RNA extracted from cells infected with mumps virus. It is best to use cells infected with a well-characterized strain of mumps, belonging to a genotype not associated with the outbreak. In addition, the use of vaccine virus as a control is not recommended since in an outbreak setting some cases may be referred for testing who have been recently vaccinated. If there is a question of contamination, this will allow sequence studies to determine the possible source of the virus.

Negative controls should include RNA extracted from uninfected cells as well as a control with water substituted for RNA. When doing large numbers of RNA extractions, it is a good idea to include an extraction control. This mock extraction will show that reagents for RNA extraction have not been contaminated by template.

2. **Avoid template contamination**. Use dedicated equipment, rooms and hoods for all pre-PCR procedures. Post amplification analysis and processing should be performed in separate rooms using dedicated equipment. Do not share equipment (including lab coats) between pre-PCR and post-PCR procedures. Use filter tips for all pipetting of pre-PCR procedures and for setting up RT-PCR reactions.

3. Use 5 ul of **RNA** per reaction. Most of the extraction protocols yield 40-50 ul of RNA. We usually extract RNA from from 100-200 uL of original viral sample or infected cells

MATERIALS:

1. Superscript kit III (Invitrogen CAT# 12574-026)
2. RNase inhibitor (pancreatic RNase inhibitor)
3. Ice bucket and/or cooling rack
4. Autoclaved PCR tubes (0.2ul, thin-walled)
5. Forward Primer (20uM working dilution prepared in RNase-free water)
6. Reverse Primer (20uM working dilution prepared in RNase-free water)
7. Synthetic mumps SH RNA with an insert can be obtained from CDC (contact Bettina Bankamp, bbankamp@cdc.gov)

INSTRUCTIONS:

1. Thaw all kit reagents except enzymes, vortex and place on ice. Keep enzymes (Superscript and RNasin) on ice at all times. Allow RNA samples to thaw on ice and keep on ice while you are setting up the reactions.
2. Label appropriate number of 0.2 ml thin-walled, reaction tubes and place in pre-chilled metal cooling rack. Keep cooling rack on ice for entire protocol.
3. Add appropriate volumes (see worksheet in Appendix 1) of reagents 1 through 5 to a pre-chilled 1.5 ml Eppendorf tube. Vortex and keep tube on ice.

4. Allow time for pre-mix contents to chill, and then add reagents 6 and 7 to pre-mix tube. Vortex and chill briefly on ice.
5. Dispense pre-mix (see worksheet) to each reaction tube.
6. Using a new, clean pipette tip for each transfer, add RNA to each tube and close the cap.
7. Spin the tubes briefly (10,000 rpm for 1 minute) in a chilled microcentrifuge and immediately return the tube to the metal cooling rack.
8. While tubes are spinning start the appropriate file the thermocycler (see parameters in Appendix 2). When the block temperature reaches 55 C, hit the pause button. With the instrument paused, transfer the reaction tubes from the metal cooling rack to the heat block of the thermocycler. Close the cover and press the resume button.
9. Reactions products should be analyzed by agarose gel electrophoresis. A 1% agarose gel is recommended (for example, see Appendix 3).

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Appendix 1
SUPERSCRIPT RT-PCR REACTION WORKSHEET:

Date:_____ Operator:_____

Component	[Final] Conc.	Vol/rxn	# rxns plus 1	Total vol.	Added Y/N
1. ETF water		16.5 uL			
2. 2X Superscript Reaction mix	1X	25 uL			
3. Forward Primer-SH1 (20 uM)	0.2 uM	0.5 uL			
4. Reverse Primer-SH2 (20 uM)	0.2 uM	0.5 uL			
Vortex and place on ice before adding enzymes					
5. Superscript Enzyme		2 uL			
6. RNase Inhibitor (optional)	20 U.	0.5 uL			
Vortex and dispense pre-mix (45 ul) into each chilled reaction tube, then add RNA samples					
7. RNA		5 uL			

Total reaction volume: 50 uL

Appendix 2
Cycling Parameters for Superscript

Step	Temperature (°C)	Duration	Number of Cycles
Reverse transcription	55	30 min	1
Denaturation	94	2 min	1
Denaturation	94	15 sec	40
Annealing	55	30 sec	
Extension	68	30 sec	
Final extension	68	5 min	1
Storage	4	hold	1

Appendix 3
Agarose gel electrophoresis of the reaction products from the standard RT-PCR for the mumps SH gene. Lane 1: molecular weight marker, lane 2: positive sample, lane 3: negative control, lane 4: positive control